UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|---|---|---|---|---|
| 10/714,508 | 11/14/2003 | Kevin Z. Qu | 034827-3002 | 9832 |

30542 7590 09/19/2007
FOLEY & LARDNER LLP
P.O. BOX 80278
SAN DIEGO, CA 92138-0278

| EXAMINER | |
|---|---|
| MUMMERT, STEPHANIE KANE | |

| ART UNIT | PAPER NUMBER |
|---|---|
| 1637 | |

| MAIL DATE | DELIVERY MODE |
|---|---|
| 09/19/2007 | PAPER |

**Please find below and/or attached an Office communication concerning this application or proceeding.**

The time period for reply, if any, is set in the attached communication.

UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
www.uspto.gov

# BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

MAILED

SEP 1 9 2007

GROUP 1600

Application Number: 10/714,508
Filing Date: November 14, 2003
Appellant(s): QU ET AL.

Richard Warburg
For Appellant

## EXAMINER'S ANSWER

This is in response to the appeal brief filed May 8, 2007 appealing from the Office action mailed September 11, 2006

**(1) Real Party in Interest**

A statement identifying by name the real party in interest is contained in the brief.

**(2) Related Appeals and Interferences**

The examiner is not aware of any related appeals, interferences, or judicial proceedings which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

**(3) Status of Claims**

The statement of the status of claims contained in the brief is correct.

**(4) Status of Amendments After Final**

The appellant's statement of the status of amendments after final rejection contained in the brief is correct.

**(5) Summary of Claimed Subject Matter**

The summary of claimed subject matter contained in the brief is correct.

**(6) Grounds of Rejection to be Reviewed on Appeal**

The appellant's statement of the grounds of rejection to be reviewed on appeal is correct.

**(7) Claims Appendix**

The copy of the appealed claims contained in the Appendix to the brief is correct.

**(8) Evidence Relied Upon**

**Lindpainter et al. (NEJM, 1995, vol. 332, no. 11, p. 706-711)**

**Lin et al. (Clinical Biochemistry, 2001, vol. 34, p. 661-666)**

**Teranishi et al. (Journal of Hypertension, 1999, vol. 17, p. 351-356)**

| | | |
|---|---|---|
| **5,736,323** | **Soubrier** | **04-1998** |

**Buck et al. (Biotechniques, 1999, vol. 27, no. 3, p. 528-536)**

**van Bockxmeer et al. (Circulation, 1995, vol. 92, p. 2066-71)**

**Hiratsuka et al. (Analytical Biochemistry, 2001, vol. 289, p. 300-303)**

**(9) Grounds of Rejection**

Applicant's arguments, see page 19, filed May 8, 2007, with respect to the rejection(s) of claim(s) 51-53, 60-61 and 71-72 under 35 U.S.C. 103 have been fully considered and are persuasive. Therefore, the rejection has been withdrawn. However, upon further consideration, a new ground(s) of rejection is made in view of Lin and Hiratsuka.

The following ground(s) of rejection are applicable to the appealed claims:

***Claim Rejections - 35 USC § 103***

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

**1.** Claims 2, 40-48, 50 and 67-70 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lindpainter et al. (NEJM, 1995, vol. 332, no. 11, p. 706-711) in view of Lin et al. (Clinical Biochemistry, 2001, vol. 34, p. 661-666). Lindpainter teaches an evaluation of the association of angiotensin converting enzyme polymorphisms with the risk of ischemic heart disease (Abstract).

With regard to claim 2 and 67, Lindpainter teaches a method of determining an angiotensin converting enzyme genotype in a sample comprising:

a) amplifying DNA from the sample with a i) first pair of flanking primers that hybridize to nucleic acid sequences flanking an ACE gene sequence, and ii) a third primer that specifically binds to said ACE gene sequence and together with one of the flanking primers forms a second pair of primers (p. 707, 'determination of ACE genotype' heading, where a first primer pair was used to amplify the D or I alleles and a third and fourth insertion specific primer pair was used to re-amplify certain samples and where it is noted that the reaction yields a 335 bp amplicon only in the presence of an I allele and no product in samples heterozygous DD); and

b) detecting a homozygous ACE genotype by the production of one or two amplification products and a heterozygous ACE genotype by the production of three amplification products (p. 707, 'determination of ACE genotype' heading, where amplicon sizes of 319 bp for D allele, 597 bp for I allele and 335 bp are all resolved, where it is noted that the reaction yields a 335 bp amplicon only in the presence of an I allele and no product in samples heterozygous DD).

With regard to claims 40 and 68, Linpainter teaches an embodiment of claims 2 and 67, wherein said amplification reaction is by polymerase chain reaction (p. 707, col. 2, 'determination of ACE genotypes' heading, where D and I alleles were identified on the basis of polymerase chain reaction amplification).

With regard to claims 41 and 69, Lindpainter teaches an embodiment of claims 2 and 67, wherein the sample is a human sample (p. 706, col. 2, 'study population' heading, where 1250 patients with ischemic heart disease were identified from the Physicians Health Study, and where these samples were submitted in EDTA-anticoagulated blood samples for storage at –80oC).

With regard to claim 42, Lindpainter teaches an embodiment of claim 41, wherein the method distinguishes between genotypes selected from the goup consisting of: insertion/insertion, insertion/deletion, deletion/deletion (Figure 1, where II, ID and DD genotypes are disclosed).

With regard to claim 43, Lindpainter teaches an embodiment of claim 41, wherein the DNA is undegraded DNA (p. 706, col. 2, 'study population' heading, where 1250 patients with ischemic heart disease were identified from the Physicians Health Study, and where these samples were submitted in EDTA-anticoagulated blood samples for storage at –80oC).

With regard to 44, Lindpainter teaches an embodiment of claim 43, wherein the sample is a tissue sample (p. 706, col. 2, 'study population' heading, where 1250 patients with ischemic heart disease were identified from the Physicians Health Study, and where these samples were submitted in EDTA-anticoagulated blood samples for storage at –80oC).

With regard to claim 45 and 46, Lindpainter teaches an embodiment of claim 44, wherein the sample is selected from the group consisting of: blood (p. 706, col. 2, 'study population' heading, where 1250 patients with ischemic heart disease were identified from the Physicians Health Study, and where these samples were submitted in EDTA-anticoagulated blood samples for storage at –80oC).

With regard to claim 47, Lindpainter teaches an embodiment of claim 41, wherein the ACE sequence resides on intron 16 of chromosome 17q23 (p. 706, col. 1, where the details of the ACE gene polymorphism are detailed).

With regard to claim 48, Lindpainter teaches an embodiment of claim 41, wherein the ACE sequence is a 287 bp nonsense DNA domain (p. 706, col. 1, where the details of the ACE gene polymorphism are detailed).

With regard to claims 50 and 70, Lindpainter teaches an embodiment of claims 41 and 67, wherein the DNA sample is from a source selected from the group consisting of: a biological fluid (p. 706, col. 2, 'study population' heading, where 1250 patients with ischemic heart disease were identified from the Physicians Health Study, and where these samples were submitted in EDTA-anticoagulated blood samples for storage at –80oC).

Regarding claim 2, Lindpainter does not teach that the amplification reaction is carried out with three separate primers in a single amplification reaction.

Lin teaches amplification using three primers simultaneously in a single amplification reaction.

With regard to claim 2, Lin teaches a method of determining an angiotensin converting enzyme (ACE) genotype in a sample comprising:
amplifying DNA in a single amplification reaction from a sample (p. 662, col. 1, 'genotyping of ACE gene I/D allele by conventional PCR' and col. 2, 'genotyping of ACE gene I/D allele by real-time PCR and melting curve analysis' heading, where ACE1-3 primers are included in a single amplification reaction).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have incorporated the genotyping analysis of the ACE genotype, with three primers included in a PCR reaction simultaneously taught by Lin into the method of ACE genotyping with Lindpainter with a reasonable expectation for success. Both Lindpainter and

Lin teach methods focused on the amplification of the 287 bp Alu insertion region within exon 16 of the ACE gene and both teach the inclusion of an insertion specific primer. The multiplexed real-time PCR amplification technique taught by Lin "takes advantage of the SYBR Green I fluorescent dye for real-time detection of PCR product and, based on the length and nucleotide contents, for the melting curve analysis of PCR products" (p. 661, col. 2 to p. 662, col. 1). Furthermore, the technique taught by Lin "provides a rapid and sensitive way for detection of ACE gene I/D polymorphism in clinical specimens" and "the applicability of real-time PCR to a high-throughput 96-well format should further reduce the overall time spent per sample and should be suitable for large-scale screening or research" (p. 665, col. 2). One of ordinary skill in the art would have recognized the benefit provided by the real-time PCR application taught by Lin and would therefore have been motivated to apply the technique to the method taught by Lindpainter with a reasonable expectation for success.

**2.** Claims 2, 40-48, 50 and 67-70 are rejected under 35 U.S.C. 103(a) as being unpatentable over Teranishi et al. (Journal of Hypertension, 1999, vol. 17, p. 351-356) in view of Lin et al. (Clinical Biochemistry, 2001, vol. 34, p. 661-666). Teranishi discloses an investigation of the association between the insertion/deletion polymorphism in the angiotensin converting enzyme and the microvascular structure of patients with non-Diabetic renal disease (Abstract).

With regard to claim 2 and 67, Teranishi teaches a method of determining an angiotensin converting enzyme genotype in a sample comprising:

a) amplifying DNA from the sample with a i) first pair of flanking primers that hybridize to nucleic acid sequences flanking an ACE gene sequence, and ii) a third primer that specifically

binds to said ACE gene sequence and together with one of the flanking primers forms a second pair of primers (p. 352, col. 1 'determination of angiotensin converting enzyme genotype' heading, where a first pair of primers results in a 190 bp fragment for the deletion, and a 490 bp fragment for the insertion, and a third insertion-specific primer results in a 408 bp PCR product in the presence of at least one insertion allele); and

b) b) detecting a homozygous ACE genotype by the production of one or two amplification products and a heterozygous ACE genotype by the production of three amplification products (p. 352, col. 1 'determination of angiotensin converting enzyme genotype' heading, where a first pair of primers results in a 190 bp fragment for the deletion, and a 490 bp fragment for the insertion, and a third insertion-specific primer results in a 408 bp PCR product in the presence of at least one insertion allele, yielding one or two products for a homozygous D or I alleles and three amplicons in the presence of a heterozygous sample).

With regard to claims 40 and 68, Teranishi teaches an embodiment of claims 2 and 67, wherein said amplification is by polymerase chain reaction (p. 352, col. 2, 'determination of angiotensiun converting enzyme genotype' heading, where the I/D, II and DD genotypes of the ACE gene were determined by PCR).

With regard to claims 41 and 69, Teranishi teaches an embodiment of claims 2 and 67, wherein the sample is a human sample (p. 352, col. 1, 'subjects' heading, where 56 patients with nondiabetic renal disease were studied and p. 352, col. 1, 'determination of angiotensin converting enzyme genotype' heading, where genomic DNA was isolated from peripheral leukocytes in a whole-blood sample).

With regard to claim 42, Teranishi teaches an embodiment of claim 3, wherein the method distinguishes between genotypes selected from the group consisting of: insertion/insertion, insertion/deletion, deletion/deletion (Table 1, where II, ID and DD genotypes are disclosed in classifying patients).

With regard to claim 43, Teranishi teaches an embodiment of claim 3, wherein the DNA is undegraded DNA (p. 352, col. 1, 'subjects' heading, where 56 patients with nondiabetic renal disease were studied and p. 352, col. 1, 'determination of angiotensin converting enzyme genotype' heading, where genomic DNA was isolated from peripheral leukocytes in a whole-blood sample).

With regard to 44, Teranishi teaches an embodiment of claim 5, wherein the sample is a tissue sample (p. 352, col. 1, 'subjects' heading, where 56 patients with nondiabetic renal disease were studied and p. 352, col. 1, 'determination of angiotensin converting enzyme genotype' heading, where genomic DNA was isolated from peripheral leukocytes in a whole-blood sample).

With regard to claim 45 and 46, Teranishi teaches an embodiment of claim 6, wherein the sample is selected from the group consisting of: blood (p. 352, col. 1, 'subjects' heading, where 56 patients with nondiabetic renal disease were studied and p. 352, col. 1, 'determination of angiotensin converting enzyme genotype' heading, where genomic DNA was isolated from peripheral leukocytes in a whole-blood sample).

With regard to claim 47, Teranishi teaches an embodiment of claim 3, wherein the ACE sequence resides on intron 16 of chromosome 17q23 (p. 351, col. 1, where the details of the ACE gene sequence and the polymorphism are disclosed).

With regard to claim 48, Teranishi teaches an embodiment of claim 3, wherein the ACE sequence is a 287 bp nonsense DNA domain (p. 351, col. 1, where the details of the ACE gene sequence and the polymorphism are disclosed).

With regard to claims 50 and 70, Teranishi teaches an embodiment of claims 2 and 67, wherein the DNA sample is from a source selected from the group consisting of: a biological fluid (p. 352, col. 1, 'subjects' heading, where 56 patients with nondiabetic renal disease were studied and p. 352, col. 1, 'determination of angiotensin converting enzyme genotype' heading, where genomic DNA was isolated from peripheral leukocytes in a whole-blood sample).

Regarding claim 2, Teranishi does not teach that the amplification reaction is carried out with three separate primers in a single amplification reaction.

Lin teaches amplification using three primers simultaneously in a single amplification reaction.

With regard to claim 2, Lin teaches a method of determining an angiotensin converting enzyme (ACE) genotype in a sample comprising:

amplifying DNA in a single amplification reaction from a sample (p. 662, col. 1, 'genotyping of ACE gene I/D allele by conventional PCR' and col. 2, 'genotyping of ACE gene I/D allele by real-time PCR and melting curve analysis' heading, where ACE1-3 primers are included in a single amplification reaction).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have incorporated the genotyping analysis of the ACE genotype, with three primers included in a PCR reaction simultaneously taught by Lin into the method of ACE genotyping with Teranishi with a reasonable expectation for success. Both Teranishi and Lin

teach methods focused on the amplification of the 287 bp Alu insertion region within exon 16 of the ACE gene and both teach the inclusion of an insertion specific primer. The multiplexed real-time PCR amplification technique taught by Lin "takes advantage of the SYBR Green I fluorescent dye for real-time detection of PCR product and, based on the length and nucleotide contents, for the melting curve analysis of PCR products" (p. 661, col. 2 to p. 662, col. 1). Furthermore, the technique taught by Lin "provides a rapid and sensitive way for detection of ACE gene I/D polymorphism in clinical specimens" and "the applicability of real-time PCR to a high-throughput 96-well format should further reduce the overall time spent per sample and should be suitable for large-scale screening or research" (p. 665, col. 2). One of ordinary skill in the art would have recognized the benefit provided by the multiplexed real-time PCR application taught by Lin and would therefore have been motivated to apply the technique to the method taught by Teranishi with a reasonable expectation for success.

3. Claims 49 and 54-59 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lindpainter et al. (NEJM, 1995, vol. 332, no. 11, p. 706-711) in view of Lin et al. (Clinical Biochemistry, 2001, vol. 34, p. 661-666) as applies to claims 2, 40-48, 50 and 67-70 above and further in view of Soubrier et al. (US Patent 5,736,323; April 1998) and Buck et al. (Biotechniques (1999) 27(3):528-536). Lindpainter teaches an evaluation of the association of angiotensin converting enzyme polymorphisms with the risk of ischemic heart disease (Abstract).

Lindpainter in view of Lin teaches all of the limitations of claims 2, 40-48, 50 and 67-70 as recited in the 103 rejection stated above.

With regard to claim 55, Lindpainter teaches an embodiment of claim 54, wherein the amplification is by polymerase chain reaction (p. 707, col. 2, 'determination of ACE genotypes' heading, where D and I alleles were identified on the basis of polymerase chain reaction amplification).

With regard to claim 56, Lindpainter teaches an embodiment of claim 55, wherein the method distinguishes between genotypes, selected from the group consisting of: insertion/insertion, insertion/deltion, deletion/deletion (Figure 1, where II, ID and DD genotypes are disclosed).

With regard to claim 57, Lindpainter teaches an embodiment of claim 55, wherein the ACE sequence resides on intron 16 of chromosome 17q23 (p. 706, col. 1, where the details of the ACE gene polymorphism are detailed).

With regard to claim 58, Lindpainter teaches an embodiment of claim 55, wherein the ACE sequence is a 287 base pair nonsense DNA domain (p. 706, col. 1, where the details of the ACE gene polymorphism are detailed).

With regard to claim 59, Lindpainter teaches an embodiment of claim 55, wherein the DNA sample is from a source selected from the group consisting of: a biological fluid (p. 706, col. 2, 'study population' heading, where 1250 patients with ischemic heart disease were identified from the Physicians Health Study, and where these samples were submitted in EDTA-anticoagulated blood samples for storage at –80oC).

Lindpainter does not teach the limitation of claims 49 and 54, c) wherein the first pair of flanking primers have the nucleic acid sequences SEQ ID NO: 1, SEQ ID NO:2 and the third primer has the nucleic acid sequence of SEQ ID NO:3.

Soubrier teaches the full-length sequence of intron 16 of the angiotensin converting enzyme and teaches the optimal location for primers for the amplification of the insertion region (Abstract, Figures 2A-B, col. 2, lines 9-50).

With regard to claim 49 and 54, Soubrier teaches an embodiment of claim 41 and 49, wherein the first pair of flanking primers have the nucleic acid sequence 5'-CCATCCTTTCTCCCATTTCTCT-3' (SEQ ID NO:1) and 5'-GGATGGTCTCGATCTCCTGA-3' (SEQ ID NO:2) and the third primer has the nucleic acid sequence 5'-CCTTAGCTCACCTCTGCTTGTAA-3' (SEQ ID NO:3) (see attached search report, where there is an exact match between nt 1416-1437 of SEQ ID 3 of Soubrier, which matches the 22 nt of SEQ ID NO:1).

In the recent court decision *In Re Deuel* 34 USPQ 2d 1210 (Fed. Cir. 1995), the Court of Appeals for the Federal Circuit determined that the existence of a general method of identifying a specific DNA does not make the specific DNA obvious. Regarding structural or functional homologs, however, the Court stated,

"Normally, a *prima facie* case of obviousness is based upon structural similiarity, i.e., an established structural relationship between a prior art compound and the claimed compound. Structural relationships may provide the requisite motivation or suggestion to modify known compounds to obtain new compounds. For example, a prior art compound may suggest its homologs because homologs often have similar properties and therefore chemists of ordinary skill would ordinarily contemplate making them to try to obtain compounds with improved properties (see page 9, paragraph 4 of attached ref)."

Since the claimed primers simply represent structural homologs, which are derived from sequences suggested by the prior art as useful for primers and probes for the detection of angiotensin converting enzyme sequences, and concerning which a biochemist of ordinary skill would have attempted to obtain alternate compounds with improved properties, the claimed primers and probes are *prima facie* obvious over the cited references in the absence of secondary

considerations.

Buck expressly provides evidence of the equivalence of primers. Specifically, Buck invited primer submissions from a number of labs (39) (page 532, column 3), with 69 different primers being submitted (see page 530, column 1). Buck also tested 95 primers spaced at 3 nucleotide intervals along the entire sequence at issue, thereby testing more than 1/3 of all possible 18-mer primers on the 300 base pair sequence (see page 530, column 1). When Buck tested each of the primers selected by the methods of the different labs, Buck found that EVERY SINGLE PRIMER worked (see page 533, column 1). Only one primer ever failed, No. 8, and that primer functioned when repeated. Further, EVERY SINGLE CONTROL PRIMER functioned as well (see page 533, column 1). Buck expressly states "The results of the empirical sequencing analysis were surprising in that nearly all of the primers yielded data of extremely high quality (page 535, column 2)." Therefore, Buck provides direct evidence that all primers would be expected to function, and in particular, all primers selected according to the ordinary criteria, however different, used by 39 different laboratories. It is particularly striking that all 95 control primers functioned, which represent 1/3 of all possible primers in the target region. This clearly shows that every primer would have a reasonable expectation of success.

Furthermore, beyond the equivalence of primers taught by Buck and the obviousness of varying the locations within a given sequence, both Lindpainter and Soubrier give specific guidance with regards to the location of specific primer sequences and these are in line with the location of the primers disclosed in the instant application. For example, Lindpainter notes that they "used an optimized primer pair to amplify the D and I alleles" (p. 707, col. 2, 'determination of ace genotype' heading). Soubrier teaches that "the invention relates more particularly to pairs of distinct fragments selected from those previously mentioned, which can be used to design primers which can be used in sequence amplification procedures" (col. 2, lines 26-31). Furthermore, Soubrier teaches that "as a result of using two primers located on either side of at

least a part of the polymorphic region, it is possible to easily visualize the difference in size due to the presence of or absence of the insertion" (col. 4, lines 7-14). As Soubrier directly states a motivation to design primers within the sequence flanking the insertion in intron 16 of the ACE gene, it would have been obvious to combine the sequence disclosed by Soubrier with the additional step taught by Lindpainter of adding an additional primer(s) designed to hybridize directly within the insertion to arrive at the claimed invention with a reasonable expectation for success.

4. Claims 62-65 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lindpainter et al. (NEJM, 1995, vol. 332, no. 11, p. 706-711) in view of Lin et al. (Clinical Biochemistry, 2001, vol. 34, p. 661-666) as applies to claims 2, 40-48, 50 and 67-70 above and further in view of van Bockxmeer et al. (Circulation, 1995, vol. 92, p. 2066-71). Lindpainter teaches an evaluation of the association of angiotensin converting enzyme polymorphisms with the risk of ischemic heart disease (Abstract).

Lindpainter teaches all of the limitations of claims 2, 40-48, 50 and 67-70 as recited in the 103 rejection stated above.

With regard to claim 62, Lindpainter teaches a method for identifying a patient with a heightened risk of suffering from a disease comprising:

a) determining the angiotensin converting enzyme (ACE) genotype in a sample from the patient by amplifying DNA from the sample with a first pair of flanking primers that hybridize to nucleic acid sequences flanking an ACE gene sequence, the presence of which indicates the presence of a first ACE gene variant, and the absence of which indicates the presence of a second gene variant, and ii) a third primer that specifically binds to said ACE gene sequence and

together with one of the flanking primers forms a second pair of primers (p. 707, 'determination of ACE genotype' heading, where a first primer pair was used to amplify the D or I alleles and a third and fourth insertion specific primer pair was used to re-amplify certain samples);

b) detecting a homozygous ACE genotype by the production of one or two amplification products and a heterozygous AcE genotype by the production of three amplification products (p. 707, 'determination of ACE genotype' heading, where amplicon sizes of 319 bp for D allele, 597 bp for I allele and 335 bp are all resolved).

With regard to claim 64, Lindpainter teaches an embodiment of claim 29, wherein the method distinguishes between genotypes selected from the group consisting of: insertion/insertion, insertion/deletion, deletion/deletion (Figure 1).

With regard to claim 65, Lindpainter teaches an embodiment of claim 29, wherein the genotype is determined by detecting the presence or absence of each of three nucleic acid products of the amplification reaction (p. 707, 'determination of ACE genotype' heading, where amplicon sizes of 319 bp for D allele, 597 bp for I allele and 335 bp are all resolved).

Lindpainter does not teach the limitation of claim 62, recited as c) correlating the ACE genotype of the patient with a treatment regimen designed to treat or prevent one or more diseases selected from the group consisting of: myocardial infarction, ischemic and idiopathic dilated cardiomyopathy, sudden death in hypertrophic cardiomyopathy, coronary artherosclerosis, and restenosis after percutaneous transluminal coronary angioplasty.

With regard to claim 62, van Bockxmeer teaches a method which correlates the ACE genotype of the patient with a treatment regimen designed to treat or prevent one or more diseases selected from the group consisting of: myocardial infarction and restenosis after

percutaneous transluminal coronary angioplasty (Table 1, where ACE genotype was correlated between angioplasty patients and control subjects, Table 2, where patient groups are characterized in groups as either with or without restenosis, Table 3, where ACE genotypes were correlated with angioplasty patients with and without restenosis, Table 4, where ACE and ApoE genotypes were correlated with angioplasty patients with and without restenosis and Table 5, where changes in lesions were compared between patients with different ACE genotypes).

With regard to claim 63, van Bockxmeer teaches an embodiment of claim 29, wherein the treatment regimen is designed to treat myocardial infarction or coronary artherosclerosis (p. 3, 'subjects and protocol' heading, where the study was restricted to patients having elective percutaneous transluminal balloon coronary angioplasty (PTCA) of a previously untreated native coronary artery).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to apply the genotyping technique taught by Lindpainter to the genotype association analysis taught by van Bockxmeer with a reasonable expectation for success. Both Lindpainter and van Bockxmeer are focused on studying the genotype of the angiotensin converting enzyme (ACE) and with the determination of any association between ACE genotype and treatment or risk of myocardial infarction. As noted by van Bockxmeer, "the ACE gene was postulated to be a candidate gene affecting the important clinical problem of restenosis after percutaneous transluminal balloon coronary angioplasty (PCTA) (Abstract). Given the obvious link between the studies conducted by Lindpainter and van Bockxmeer, it would have been obvious to substitute the genotyping method taught by Lindpainter to the method taught by van Bockxmeer with a reasonable expectation for success.

5. Claim 66 is rejected under 35 U.S.C. 103(a) as being unpatentable over Lindpainter et al. (NEJM, 1995, vol. 332, no. 11, p. 706-711) in view of Lin et al. (Clinical Biochemistry, 2001, vol. 34, p. 661-666) as applies to claims 2, 40-48, 50 and 67-70 above and further in view of van Bockxmeer et al. (Circulation, 1995, vol. 92, p. 2066-71) and further in view of Soubrier et al. (US Patent 5,736,323; April 1998) and Buck et al. (Biotechniques (1999) 27(3):528-536). Lindpainter teaches an evaluation of the association of angiotensin converting enzyme polymorphisms with the risk of ischemic heart disease (Abstract).

Lindpainter teaches all of the limitations of claims 2, 40-48, 50 and 67-70 as recited in the 103 rejection stated above. Lindpainter does not teach the limitation of claim 66 wherein the first pair of flanking primers have the nucleic acid sequences SEQ ID NO: 1, SEQ ID NO:2 and the third primer has the nucleic acid sequence of SEQ ID NO:3.

Soubrier teaches the full-length sequence of intron 16 of the angiotensin converting enzyme and teaches the optimal location for primers for the amplification of the insertion region (Abstract, Figures 2A-B, col. 2, lines 9-50).

With regard to claim 66, Soubrier teaches an embodiment of claim 63, wherein the pair of flanking primers have the nucleic acid sequences 5'-CCATCCTTTCTCCCATTTCTCT-3' (SEQ ID NO:1) and 5'-GGATGGTCTCGATCTCCTGA-3' (SEQ ID NO:2) and the third primer has the nucleic acid sequence 5'-CCTTAGCTCACCTCTGCTTGTAA-3' (SEQ ID NO:3) (see attached search report, where there is an exact match between nt 1416-1437 of SEQ ID 3 of Soubrier, which matches the 22 nt of SEQ ID NO:1).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to adjust and optimize the location of the primers taught by Lindpainter, including those disclosed within the instant application and disclosed generally by Soubrier.

In the recent court decision *In Re Deuel* 34 USPQ 2d 1210 (Fed. Cir. 1995), the Court of Appeals for the Federal Circuit determined that the existence of a general method of identifying a specific DNA does not make the specific DNA obvious. Regarding structural or functional homologs, however, the Court stated,

"Normally, a *prima facie* case of obviousness is based upon structural similiarity, i.e., an established structural relationship between a prior art compound and the claimed compound. Structural relationships may provide the requisite motivation or suggestion to modify known compounds to obtain new compounds. For example, a prior art compound may suggest its homologs because homologs often have similar properties and therefore chemists of ordinary skill would ordinarily contemplate making them to try to obtain compounds with improved properties (see page 9, paragraph 4 of attached ref)."

Since the claimed primers simply represent structural homologs, which are derived from sequences suggested by the prior art as useful for primers and probes for the detection of angiotensin converting enzyme sequences, and concerning which a biochemist of ordinary skill would have attempted to obtain alternate compounds with improved properties, the claimed primers and probes are *prima facie* obvious over the cited references in the absence of secondary considerations.

Buck expressly provides evidence of the equivalence of primers. Specifically, Buck invited primer submissions from a number of labs (39) (page 532, column 3), with 69 different primers being submitted (see page 530, column 1). Buck also tested 95 primers spaced at 3 nucleotide intervals along the entire sequence at issue, thereby testing more than 1/3 of all possible 18-mer primers on the 300 base pair sequence (see page 530, column 1). When Buck tested each of the primers selected by the methods of the different labs, Buck found that EVERY SINGLE PRIMER worked (see page 533, column 1). Only one primer ever failed, No. 8, and

that primer functioned when repeated. Further, EVERY SINGLE CONTROL PRIMER functioned as well (see page 533, column 1). Buck expressly states "The results of the empirical sequencing analysis were surprising in that nearly all of the primers yielded data of extremely high quality (page 535, column 2)." Therefore, Buck provides direct evidence that all primers would be expected to function, and in particular, all primers selected according to the ordinary criteria, however different, used by 39 different laboratories. It is particularly striking that all 95 control primers functioned, which represent 1/3 of all possible primers in the target region. This clearly shows that every primer would have a reasonable expectation of success.

Furthermore, beyond the equivalence of primers taught by Buck and the obviousness of varying the locations within a given sequence, both Lindpainter and Soubrier give specific guidance with regards to the location of specific primer sequences and these are in line with the location of the primers disclosed in the instant application. For example, Lindpainter notes that they "used an optimized primer pair to amplify the D and I alleles" (p. 707, col. 2, 'determination of ace genotype' heading). Soubrier teaches that "the invention relates more particularly to pairs of distinct fragments selected from those previously mentioned, which can be used to design primers which can be used in sequence amplification procedures" (col. 2, lines 26-31). Furthermore, Soubrier teaches that "as a result of using two primers located on either side of at least a part of the polymorphic region, it is possible to easily visualize the difference in size due to the presence of or absence of the insertion" (col. 4, lines 7-14). As Soubrier directly states a motivation to design primers within the sequence flanking the insertion in intron 16 of the ACE gene, it would have been obvious to combine the sequence disclosed by Soubrier with the additional step taught by Lindpainter of adding an additional primer(s) designed to hybridize directly within the insertion to arrive at the claimed invention with a reasonable expectation for success.

**New Grounds of Rejection**

**6.** Claims 2, 40-48, 50 and 67-70 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lin et al. (Clinical Biochemistry, 2001, vol. 34, p. 661-666) in view of Hiratsuka et al. (Analytical Biochemistry, 2001, vol. 289, p. 300-303). Lin teaches amplification using three primers simultaneously in a single amplification reaction for the determination of angiotensin converting enzyme genotype (Abstract).

With regard to claim 2 and 67, Lin teaches a method of determining an angiotensin converting enzyme genotype in a sample comprising:

a) amplifying DNA from the sample with a i) first pair of flanking primers that hybridize to nucleic acid sequences flanking an ACE gene sequence, and ii) a third primer that specifically binds to said ACE gene sequence and together with one of the flanking primers forms a second pair of primers (p. 662, col. 2, 'genotyping of ACEgene I/D allele by real-time PCR and melting curve analysis heading' where there are three primers present, ACE1, ACE2, ACE3, see also Figure 1, where the locations of these primers are depicted and where ACE1 and ACE3 represent a first pair of flanking primers and where ACE2 represents a third primer that binds to ACE gene sequence and forms a second pair of primers); and

b) detecting a homozygous ACE genotype by the production of one or two amplification products (Figure 3, where homozygous ACE genotypes are detected by the production of one or two amplification products, see melting peaks for the I/I or D/D genotypes).

With regard to claims 40 and 68, Lin teaches an embodiment of claims 2 and 67, wherein said amplification reaction is by polymerase chain reaction (p. 662, col. 2, 'genotyping of ACEgene I/D allele by real-time PCR and melting curve analysis heading' where there are three

primers present, ACE1, ACE2, ACE3 and these primers are used in PCR amplification reactions).

With regard to claims 41 and 69, Lin teaches an embodiment of claims 2 and 67, wherein the sample is a human sample (p. 662, col. 1, 'isolation of genomic DNA' heading, where DNA was isolated from blood samples obtained from the Department of Internal Medicine, National Taiwan University; see also p. 661, where the role of this enzyme in human disorders is described in detail).

With regard to claim 42, Lin teaches an embodiment of claim 41, wherein the method distinguishes between genotypes selected from the goup consisting of: insertion/insertion, insertion/deletion, deletion/deletion (Figure 3, where the three genotypes are distinguished, I/I, I/D and D/D alleles are genotyped).

With regard to claim 43, Lin teaches an embodiment of claim 41, wherein the DNA is undegraded DNA (p. 662, col. 1, 'isolation of genomic DNA' heading, where DNA was isolated from blood samples obtained from the Department of Internal Medicine, National Taiwan University).

With regard to 44, Lin teaches an embodiment of claim 43, wherein the sample is a tissue sample (p. 662, col. 1, 'isolation of genomic DNA' heading, where DNA was isolated from blood samples obtained from the Department of Internal Medicine, National Taiwan University).

With regard to claim 45 and 46, Lin teaches an embodiment of claim 44, wherein the sample is selected from the group consisting of: blood (p. 662, col. 1, 'isolation of genomic DNA' heading, where DNA was isolated from blood samples obtained from the Department of Internal Medicine, National Taiwan University).

With regard to claim 47, Lin teaches an embodiment of claim 41, wherein the ACE sequence resides on Intron 16 of chromosome 17q23 (p. 661, col. 1, where the ACE genotype is based on a polymorphism in Intron 16 on chromosome 17).

With regard to claim 48, Lin teaches an embodiment of claim 41, wherein the ACE sequence is a 287 bp nonsense DNA domain (Figure 1, where the grayed region represents the insertion sequence of the ACE gene; p. 1, col. 1, where the 287 bp Alu sequence is described).

With regard to claims 50 and 70, Lin teaches an embodiment of claims 41 and 67, wherein the DNA sample is from a source selected from the group consisting of: a biological fluid (p. 662, col. 1, 'isolation of genomic DNA' heading, where DNA was isolated from blood samples obtained from the Department of Internal Medicine, National Taiwan University).

Regarding claim 2, Lin does not teach the detection of ACE genotype by the production of three amplification products.

With regard to claim 1, Hiratsuka in view of Lin teaches the detection of heterozygous ACE genotype by the production of three amplification products (p. 301, col. 1, where the amplification using the flanking primers resulted in two amplicons 373 bp, for the insertion allele and 85 bp for the deletion allele; see also Figure 1, and where combining these amplicons with the amplicons provided by Lin would produce three amplification products in the heterozygote - 2 products for the I allele and 1 for the D allele).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have extended the genotyping analysis of the ACE genotype, with three primers included in a PCR reaction simultaneously taught by Lin to incorporate the third amplicon comprising the product of the ACE1 and ACE3 primers in the presence of the 287 bp

Alu insertion, resulting in an amplicon of 373 basepairs as taught by Hiratsuka (p. 301, col. 1). Both Lin and Hiratsuka teach methods focused on the amplification of the 287 bp Alu insertion region within exon 16 of the ACE gene and both teach the inclusion of an insertion specific primer. The multiplexed real-time PCR amplification technique taught by Lin genotypes the samples using three primers, however only detects two of the three potential amplification products - the deletion allele, a product of the amplification using ACE1 and ACE3 when the insertion is not present, resulting in an amplicon of 84 base pairs in length and the insertion allele, a product of the ACE2 (insertion specific) primer and ACE3, resulting in an amplicon of 65 base pairs in length.

Hiratsuka teaches a similar method of genotype analysis and teaches very similar flanking primers which amplify the same general region of the ACE gene sequence. Unlike Lin, Hiratsuka detects two amplicons, both products of the 'ACE1' and 'ACE3' primers - an insertion allele, where the full length product of the flanking primers produces an amplicon of 373 base pairs in length when the insertion is present, and a deletion allele, where the product of the flanking primer produces an amplicon of 85 base pairs in length when the insertion is not present.

Therefore, while Lin teaches the three primers and does not detect the third amplicon, Hiratsuka teaches that the product of these flanking primers would be expected to detect a product when the insertion is present that is 387 base pairs in length. Furthermore, as taught by Hiratsuka, it would be expected that this larger amplicon, when analyzed using melting curve analysis would melt in a pattern that would produce three peaks (Figure 1; p. 301, col. 2, where homozygous PCR of the insertion type showed three peaks at 80.7°C, 85.8 °C, 88.8 °C). Finally,

considering the combined teachings of Lin and Hiratsuka, it would have been prima facie obvious to one of ordinary skill in the art that the placement of the primers taught by Lin, using the conditions and primer concentrations taught by Hiratsuka would produce a set of amplicons wherein the presence of three amplicons is indicative of a heterozygous genotype with a reasonable expectation for success.

Finally, when Lin and Hiratsuka are combined, each of the primers and the three amplicons produced by these primers are taught. When these amplicons are produced through a combination of the methods taught by both Lin and Hiratsuka, a characteristic number of amplicons will be produced for each possible genotype - two amplicons for I/I homozygotes, one for D/D homozygotes and three for I/D heterozygotes. Therefore it would have been obvious to combine these separate primers in order to achieve the predictable result of three amplicons produced by three pairs of primers to arrive at the claimed invention.

7. Claims 49 and 51-61 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lin et al. (Clinical Biochemistry, 2001, vol. 34, p. 661-666) in view of Hiratsuka et al. (Analytical Biochemistry, 2001, vol. 289, p. 300-303) as applies to claims 2, 40-48, 50 and 67-70 above and further in view of Soubrier et al. (US Patent 5,736,323; April 1998) and Buck et al. (Biotechniques (1999) 27(3):528-536). Lin teaches amplification using three primers simultaneously in a single amplification reaction for the determination of angiotensin converting enzyme genotype (Abstract).

Lin in view of Hiratsuka teaches all of the limitations of claims 2, 40-48, 50 and 67-70 as recited in the 103 rejection stated above.

With regard to claim 51, 52 and 60, Lin teaches an embodiment of claim 41, 49 and 55, wherein the nucleic acid products consist of a first nucleic acid fragment of approximately 123 (D allele) base pairs, a second nucleic acid fragment of approximately 157 (I allele specific primer pair) base pairs (p. 663, where the insertion allele of Lin comprises 65 base pairs which is approximately 157 base pairs and where the deletion allele comprises 84 base pairs which is approximately 123 base pairs).

With regard to claim 53 and 61, Lin teaches an embodiment of claim 52 and 60, wherein:
a) when the first nucleic acid fragment (123) is not present and the second (157) fragments are present, the genotype is I/I (p. 663, where the insertion allele of Lin comprises 65 base pairs which is approximately 157 base pairs);
b) when the first, second nucleic acid fragments are present, the genotype is I/D (p. 663, where the insertion allele of Lin comprises 65 base pairs which is approximately 157 base pairs and where the deletion allele comprises 84 base pairs which is approximately 123 base pairs ); and
c) when the first nucleic acid fragment is present and the second and the third nucleic acid fragments are not present, the genotype is D/D (p. 663, where the deletion allele of Lin comprises 85 bp which is approximately 123 basepairs).

With regard to claim 55, Lin teaches an embodiment of claim 54, wherein the amplification is by polymerase chain reaction (p. 662, col. 2, 'genotyping of ACEgene I/D allele by real-time PCR and melting curve analysis heading' where there are three primers present, ACE1, ACE2, ACE3 and these primers are used in PCR amplification reactions).

With regard to claim 56, Lin teaches an embodiment of claim 55, wherein the method distinguishes between genotypes, selected from the group consisting of: insertion/insertion,

insertion/deltion, deletion/deletion (Figure 3, where the three genotypes are distinguished, I/I, I/D and D/D alleles are genotyped).

With regard to claim 57, Lin teaches an embodiment of claim 55, wherein the ACE sequence resides on intron 16 of chromosome 17q23 (p. 661, col. 1, where the ACE genotype is based on a polymorphism in Intron 16 on chromosome 17).

With regard to claim 58, Lin teaches an embodiment of claim 55, wherein the ACE sequence is a 287 base pair nonsense DNA domain (Figure 1, where the grayed region represents the insertion sequence of the ACE gene; p. 1, col. 1, where the 287 bp Alu sequence is described).

With regard to claim 59, Lin teaches an embodiment of claim 55, wherein the DNA sample is from a source selected from the group consisting of: a biological fluid (p. 662, col. 1, 'isolation of genomic DNA' heading, where DNA was isolated from blood samples obtained from the Department of Internal Medicine, National Taiwan University).

Regarding claim 51-53, 60-61, Lin does not teach the presence of a third amplicon.

With regard to claim 51-52 and 60, Hiratsuka teaches nucleic acid products consisting of a third nucleic acid fragment of 410 base pairs (p. 301, col. 1, where the amplification using the flanking primers resulted in a 373 bp for the insertion allele, which is approximately 410 base pairs).

With regard to claim 53 and 61, Hiratsuka in view of Lin teaches wherein:

a) when the first nucleic acid fragment is not present and the second and third nucleic acid fragment is present, the genotype is I/I (p. 301, col. 1, where the amplification using the flanking primers resulted in a 373 bp amplicon, for the insertion allele and where these flanking primers

overlap with the forward and reverse flanking primers disclosed by Lin and when this product is combined with the 65 bp product for the I allele disclosed by Lin, a second and third amplicon is present for samples with I/I genotype); and

b) when the first, second and third nucleic acid fragments are present, the genotype is I/D (p. 301, col. 1 of Hiratsuka, where the amplification using the flanking primers resulted in a 373 bp amplicon, for the I allele and where these flanking primers overlap with the forward and reverse flanking primers disclosed by Lin. When this product is combined with the 65 bp product for the I allele and 84 bp for the D allele as disclosed by Lin, results in three amplicons for the I/D genotype).

Lin does not teach the limitation of claims 49 and 54, c) wherein the first pair of flanking primers have the nucleic acid sequences SEQ ID NO: 1, SEQ ID NO:2 and the third primer has the nucleic acid sequence of SEQ ID NO:3, however these primer sequences are located very nearby the primer sequences disclosed in SEQ ID NO:1-3. Particularly for SEQ ID NO:1, Lin teaches the identical sequence but includes an additional A at the 3' end of the sequence as compared to SEQ ID NO:1 (see Figure 1, where the primer locations of Lin are depicted in sequence specifically).

Soubrier teaches the full-length sequence of intron 16 of the angiotensin converting enzyme and teaches the optimal location for primers for the amplification of the insertion region (Abstract, Figures 2A-B, col. 2, lines 9-50).

With regard to claim 49 and 54, Soubrier teaches an embodiment of claim 41 and 49, wherein the first pair of flanking primers have the nucleic acid sequence 5'-CCATCCTTTCTCCCATTTCTCT-3' (SEQ ID NO:1) and 5'-GGATGGTCTCGATCTCCTGA-

3' (SEQ ID NO:2) and the third primer has the nucleic acid sequence 5'-CCTTAGCTCACCTCTGCTTGTAA-3' (SEQ ID NO:3) (see attached search report, where there is an exact match between nt 1416-1437 of SEQ ID 3 of Soubrier, which matches the 22 nt of SEQ ID NO:1).

Regarding claims 51-52 and 60, while Lin does not disclose each of the specific amplicon sizes recited within the claim, it would have been obvious to one of ordinary skill in the art that if the primer locations were changed the amplicon size would change accordingly. Thus, an ordinary practitioner would have recognized that the results optimizable variables of time, product amount and sequence length could be adjusted to maximize the desired results. As noted in *In re Aller*, 105 USPQ 233 at 235,

> More particularly, where the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation.

Routine optimization is not considered inventive and no evidence has been presented that the selection of specific amplicon sizes was other than routine, that the products resulting from the optimization have any unexpected properties, or that the results should be considered unexpected in any way as compared to the closest prior art.

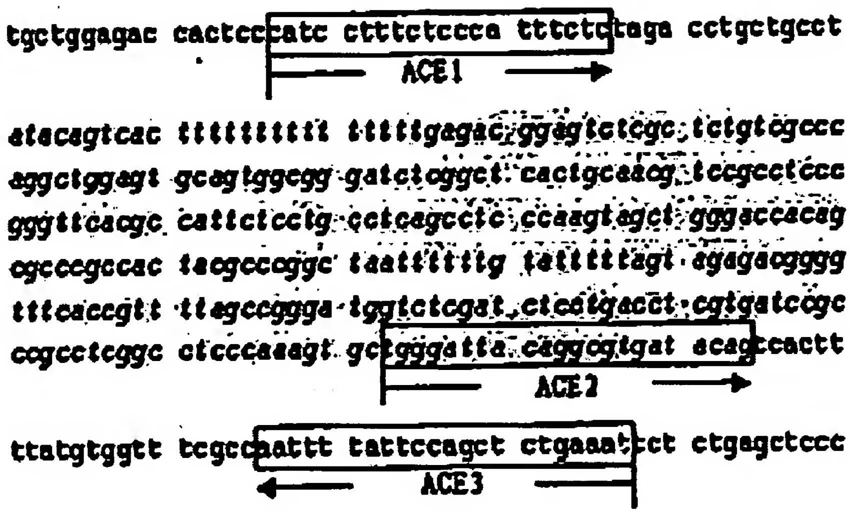


**Fig. 1. Design of primers for real-time PCR detection of ACE gene insertion/deletion polymorphism. The sequences and relative position of primers ACE1, ACE2, and ACE3 were boxed. The gray-filled region was the intron 16 sequences of ACE gene.**

Considering that this is a known region of interest of limited length (see Figure inserted above, Lin, Figure 1), the primers must be selected within this specific region, including the flanking primers and particularly the insertion specific primer site (ACE2 above). It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have designed primers within these defined regions to obtain amplicons of a predetermined length. The process of designing primers would have been obvious to one of ordinary skill in the art at the time the invention was made in order to achieve the predictable result of obtaining an amplicon of the desired size.

Furthermore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to adjust and optimize the location of the primers taught by Lindpainter, including those disclosed within the instant application and disclosed generally by Soubrier.

In the recent court decision *In Re Deuel* 34 USPQ 2d 1210 (Fed. Cir. 1995), the Court of Appeals for the Federal Circuit determined that the existence of a general method of identifying a specific DNA does not make the specific DNA obvious. Regarding structural or functional homologs, however, the Court stated,

"Normally, a *prima facie* case of obviousness is based upon structural similiarity, i.e., an established structural relationship between a prior art compound and the claimed compound. Structural relationships may provide the requisite motivation or suggestion to modify known compounds to obtain new compounds. For example, a prior art compound may suggest its homologs because homologs often have similar properties and therefore chemists of ordinary skill would ordinarily contemplate making them to try to obtain compounds with improved properties (see page 9, paragraph 4 of attached ref)."

Since the claimed primers simply represent structural homologs, which are derived from sequences suggested by the prior art as useful for primers and probes for the detection of angiotensin converting enzyme sequences, and concerning which a biochemist of ordinary skill would have attempted to obtain alternate compounds with improved properties, the claimed primers and probes are *prima facie* obvious over the cited references in the absence of secondary considerations.

Buck expressly provides evidence of the equivalence of primers. Specifically, Buck invited primer submissions from a number of labs (39) (page 532, column 3), with 69 different primers being submitted (see page 530, column 1). Buck also tested 95 primers spaced at 3 nucleotide intervals along the entire sequence at issue, thereby testing more than 1/3 of all possible 18-mer primers on the 300 base pair sequence (see page 530, column 1). When Buck tested each of the primers selected by the methods of the different labs, Buck found that EVERY SINGLE PRIMER worked (see page 533, column 1). Only one primer ever failed, No. 8, and that primer functioned when repeated. Further, EVERY SINGLE CONTROL PRIMER functioned as well (see page 533, column 1). Buck expressly states "The results of the empirical sequencing analysis were surprising in that nearly all of the primers yielded data of extremely high quality (page 535, column 2)." Therefore, Buck provides direct evidence that all primers

would be expected to function, and in particular, all primers selected according to the ordinary criteria, however different, used by 39 different laboratories. It is particularly striking that all 95 control primers functioned, which represent 1/3 of all possible primers in the target region. This clearly shows that every primer would have a reasonable expectation of success.

Furthermore, beyond the equivalence of primers taught by Buck and the obviousness of varying the locations within a given sequence, Lin, Hiratsuka and Soubrier give specific guidance with regards to the location of specific primer sequences and these are in line with the location of the primers disclosed in the instant application. For example, Lin notes "Real-time PCR was performed with three ACE gene primers (ACE1, ACE2, and ACE3, Fig. 1) in the presence of SYBR green I dye" (p. 662, col. 1). While Hiratsuka teaches "we conjectured that it might also be possible to detect mutations with SYBR Green I in small PCR products by differences in the melting behavior of high and low molecules. This would enable the screening of large genes for the presence of insertion/deletion by analyzing many overlapping PCR products (p. 301, col. 1)". Soubrier teaches that "the invention relates more particularly to pairs of distinct fragments selected from those previously mentioned, which can be used to design primers which can be used in sequence amplification procedures" (col. 2, lines 26-31). Furthermore, Soubrier teaches that "as a result of using two primers located on either side of at least a part of the polymorphic region, it is possible to easily visualize the difference in size due to the presence of or absence of the insertion" (col. 4, lines 7-14). As Soubrier directly states a motivation to design primers within the sequence flanking the insertion in intron 16 of the ACE gene, it would have been obvious to combine the sequence disclosed by Soubrier with the additional step taught by Lin of adding an additional primer(s) designed to hybridize directly within the insertion to arrive at the claimed invention with a reasonable expectation for success.

8. Claims 62-65 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lin et al. (Clinical Biochemistry, 2001, vol. 34, p. 661-666) in view of Hiratsuka et al. (Analytical Biochemistry, 2001, vol. 289, p. 300-303) as applies to claims 2, 40-48, 50 and 67-70 above and further in view of van Bockxmeer et al. (Circulation, 1995, vol. 92, p. 2066-71). Lin teaches amplification using three primers simultaneously in a single amplification reaction for the determination of angiotensin converting enzyme genotype (Abstract).

Lin in view of Hiratsuka teaches all of the limitations of claims 2, 40-48, 50 and 67-70 as recited in the 103 rejection stated above.

With regard to claim 62, Lin teaches a method for identifying a patient with a heightened risk of suffering from a disease comprising:

a) determining the angiotensin converting enzyme (ACE) genotype in a sample from the patient by amplifying DNA from the sample with a first pair of flanking primers that hybridize to nucleic acid sequences flanking an ACE gene sequence, the presence of which indicates the presence of a first ACE gene variant, and the absence of which indicates the presence of a second gene variant, and ii) a third primer that specifically binds to said ACE gene sequence and together with one of the flanking primers forms a second pair of primers (p. 662, col. 2, 'genotyping of ACEgene I/D allele by real-time PCR and melting curve analysis heading' where there are three primers present, ACE1, ACE2, ACE3, see also Figure 1, where the locations of these primers are depicted and where ACE1 and ACE3 represent a first pair of flanking primers and where ACE2 represents a third primer that binds to ACE gene sequence and forms a second pair of primers);

b) detecting a homozygous ACE genotype by the production of one or two amplification products and a heterozygous ACE genotype by the production of three amplification products (Figure 3, where homozygous ACE genotypes are detected by the production of one or two amplification products, see melting peaks for the I/I or D/D genotypes).

With regard to claim 64, Lin teaches an embodiment of claim 29, wherein the method distinguishes between genotypes selected from the group consisting of: insertion/insertion, insertion/deletion, deletion/deletion (Figure 3, where the three genotypes are distinguished, I/I, I/D and D/D alleles are genotyped).

With regard to claim 65, Lin teaches an embodiment of claim 29, wherein the genotype is determined by detecting the presence or absence of nucleic acid products of the amplification reaction (Figure 3, where the three genotypes are distinguished, I/I, I/D and D/D alleles are genotyped).

Regarding claim 65, Lin does not teach detection of the presence or absence of three nucleic acid products.

With regard to claim 65, Hiratsuka in view of Lin teaches an embodiment of claim 29, wherein the genotype is determined by detecting the presence or absence of each of three nucleic acid products of the amplification reaction (p. 301, col. 1 of Hiratsuka, where the amplification using the flanking primers resulted in a 373 bp amplicon, for the I allele and where these flanking primers overlap with the forward and reverse flanking primers disclosed by Lin. When this amplicon is combined with the 65 bp product for the I allele and 84 bp for the D allele as disclosed by Lin, three amplicons for the I/D genotype are detected).

Neither Lin nor Hiratsuka teach the limitation of claim 62, recited as c) correlating the ACE genotype of the patient with a treatment regimen designed to treat or prevent one or more diseases selected from the group consisting of: myocardial infarction, ischemic and idiopathic dilated cardiomyopathy, sudden death in hypertrophic cardiomyopathy, coronary artherosclerosis, and restenosis after percutaneous transluminal coronary angioplasty.

With regard to claim 62, van Bockxmeer teaches a method which correlates the ACE genotype of the patient with a treatment regimen designed to treat or prevent one or more diseases selected from the group consisting of: myocardial infarction and restenosis after percutaneous transluminal coronary angioplasty (Table 1, where ACE genotype was correlated between angioplasty patients and control subjects, Table 2, where patient groups are characterized in groups as either with or without restenosis, Table 3, where ACE genotypes were correlated with angioplasty patients with and without restenosis, Table 4, where ACE and ApoE genotypes were correlated with angioplasty patients with and without restenosis and Table 5, where changes in lesions were compared between patients with different ACE genotypes).

With regard to claim 63, van Bockxmeer teaches an embodiment of claim 29, wherein the treatment regimen is designed to treat myocardial infarction or coronary artherosclerosis (p. 3, 'subjects and protocol' heading, where the study was restricted to patients having elective percutaneous transluminal balloon coronary angioplasty (PTCA) of a previously untreated native coronary artery).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to apply the genotyping technique taught by Lin and Hiratsuka to the genotype association analysis taught by van Bockxmeer with a reasonable expectation for

success. Lin, Hiratsuka and van Bockxmeer are focused on studying the genotype of the angiotensin converting enzyme (ACE) and with the determination of any association between ACE genotype and treatment or risk of myocardial infarction. As noted by van Bockxmeer, "the ACE gene was postulated to be a candidate gene affecting the important clinical problem of restenosis after percutaneous transluminal balloon coronary angioplasty (PCTA) (Abstract). Given the obvious link between the studies conducted by Lin, Hiratsuka and van Bockxmeer, it would have been obvious to substitute the genotyping method taught by Lin and Hiratsuka to the method taught by van Bockxmeer with a reasonable expectation for success.

9. Claim 66 is rejected under 35 U.S.C. 103(a) as being unpatentable over Lin et al. (Clinical Biochemistry, 2001, vol. 34, p. 661-666) in view of Hiratsuka et al. (Analytical Biochemistry, 2001, vol. 289, p. 300-303) as applies to claims 2, 40-48, 50 and 67-70 above and further in view of van Bockxmeer et al. (Circulation, 1995, vol. 92, p. 2066-71) and further in view of Soubrier et al. (US Patent 5,736,323; April 1998) and Buck et al. (Biotechniques (1999) 27(3):528-536). Lin teaches amplification using three primers simultaneously in a single amplification reaction for the determination of angiotensin converting enzyme genotype (Abstract).

Lin in view of Hiratsuka teaches all of the limitations of claims 2, 40-48, 50 and 67-70 as recited in the 103 rejection stated above. Neither Lin nor Hiratsuka teach the limitation of claim 66 wherein the first pair of flanking primers have the nucleic acid sequences SEQ ID NO: 1, SEQ ID NO:2 and the third primer has the nucleic acid sequence of SEQ ID NO:3.

Soubrier teaches the full-length sequence of intron 16 of the angiotensin converting enzyme and teaches the optimal location for primers for the amplification of the insertion region (Abstract, Figures 2A-B, col. 2, lines 9-50).

With regard to claim 66, Soubrier teaches an embodiment of claim 63, wherein the pair of flanking primers have the nucleic acid sequences 5'-CCATCCTTTCTCCCATTTCTCT-3' (SEQ ID NO:1) and 5'-GGATGGTCTCGATCTCCTGA-3' (SEQ ID NO:2) and the third primer has the nucleic acid sequence 5'-CCTTAGCTCACCTCTGCTTGTAA-3' (SEQ ID NO:3) (see attached search report, where there is an exact match between nt 1416-1437 of SEQ ID 3 of Soubrier, which matches the 22 nt of SEQ ID NO:1).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to adjust and optimize the location of the primers taught by Lindpainter, including those disclosed within the instant application and disclosed generally by Soubrier.

In the recent court decision *In Re Deuel* 34 USPQ 2d 1210 (Fed. Cir. 1995), the Court of Appeals for the Federal Circuit determined that the existence of a general method of identifying a specific DNA does not make the specific DNA obvious. Regarding structural or functional homologs, however, the Court stated,

"Normally, a *prima facie* case of obviousness is based upon structural similiarity, i.e., an established structural relationship between a prior art compound and the claimed compound. Structural relationships may provide the requisite motivation or suggestion to modify known compounds to obtain new compounds. For example, a prior art compound may suggest its homologs because homologs often have similar properties and therefore chemists of ordinary skill would ordinarily contemplate making them to try to obtain compounds with improved properties (see page 9, paragraph 4 of attached ref)."

Since the claimed primers simply represent structural homologs, which are derived from sequences suggested by the prior art as useful for primers and probes for the detection of angiotensin converting enzyme sequences, and concerning which a biochemist of ordinary skill

would have attempted to obtain alternate compounds with improved properties, the claimed primers and probes are *prima facie* obvious over the cited references in the absence of secondary considerations.

Buck expressly provides evidence of the equivalence of primers. Specifically, Buck invited primer submissions from a number of labs (39) (page 532, column 3), with 69 different primers being submitted (see page 530, column 1). Buck also tested 95 primers spaced at 3 nucleotide intervals along the entire sequence at issue, thereby testing more than 1/3 of all possible 18-mer primers on the 300 base pair sequence (see page 530, column 1). When Buck tested each of the primers selected by the methods of the different labs, Buck found that EVERY SINGLE PRIMER worked (see page 533, column 1). Only one primer ever failed, No. 8, and that primer functioned when repeated. Further, EVERY SINGLE CONTROL PRIMER functioned as well (see page 533, column 1). Buck expressly states "The results of the empirical sequencing analysis were surprising in that nearly all of the primers yielded data of extremely high quality (page 535, column 2)." Therefore, Buck provides direct evidence that all primers would be expected to function, and in particular, all primers selected according to the ordinary criteria, however different, used by 39 different laboratories. It is particularly striking that all 95 control primers functioned, which represent 1/3 of all possible primers in the target region. This clearly shows that every primer would have a reasonable expectation of success.

Furthermore, beyond the equivalence of primers taught by Buck and the obviousness of varying the locations within a given sequence, Lin, Hiratsuka and Soubrier give specific guidance with regards to the location of specific primer sequences and these are in line with the location of the primers disclosed in the instant application. For example, Lin notes "Real-time PCR was performed with three ACE gene primers (ACE1, ACE2, and ACE3, Fig. 1) in the presence of SYBR green I dye" (p. 662, col. 1). While Hiratsuka teaches "we conjectured that it might also be possible to detect mutations with SYBR Green I in small PCR products by

differences in the melting behavior of high and low molecules. This would enable the screening of large genes for the presence of insertion/deletion by analyzing many overlapping PCR products (p. 301, col. 1)". Soubrier teaches that "the invention relates more particularly to pairs of distinct fragments selected from those previously mentioned, which can be used to design primers which can be used in sequence amplification procedures" (col. 2, lines 26-31). Furthermore, Soubrier teaches that "as a result of using two primers located on either side of at least a part of the polymorphic region, it is possible to easily visualize the difference in size due to the presence of or absence of the insertion" (col. 4, lines 7-14). As Soubrier directly states a motivation to design primers within the sequence flanking the insertion in intron 16 of the ACE gene, it would have been obvious to combine the sequence disclosed by Soubrier with the additional step taught by Lin of adding an additional primer(s) designed to hybridize directly within the insertion to arrive at the claimed invention with a reasonable expectation for success.

10. Claims 71-72 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lin et al. (Clinical Biochemistry, 2001, vol. 34, p. 661-666) in view of Hiratsuka et al. (Analytical Biochemistry, 2001, vol. 289, p. 300-303) as applies to claims 2, 40-48, 50 and 67-70. Lin teaches amplification using three primers simultaneously in a single amplification reaction for the determination of angiotensin converting enzyme genotype (Abstract).

Lin and Hiratsuka teach all of the limitations of claims 2, 40-48, 50 and 67-70 as recited in the 103 rejection stated above.

With regard to claim 71 and 72, Lin teaches an embodiment of claim 67, wherein the nucleic acid products consist of a first nucleic acid fragment of approximately 123 base pairs, a second nucleic acid fragment of approximately 157 base pairs, and a third nucleic acid fragment of approximately 410 base pairs (p. 663, where the insertion allele of Lin comprises 65 base pairs

which is approximately 157 base pairs and where the deletion allele comprises 84 base pairs which is approximately 123 base pairs).

Regarding claims 71-71, Lin does not teach the presence of a third amplicon.

With regard to claims 71-72, Hiratsuka teaches nucleic acid products consisting of a third nucleic acid fragment of 410 base pairs (p. 301, col. 1, where the amplification using the flanking primers resulted in a 373 bp, for the insertion allele).

Regarding claims 71-72, while Lin does not disclose each of the specific amplicon sizes recited within the claim, it would have been obvious to one of ordinary skill in the art that if the primer locations were changed the amplicon size would change accordingly. Thus, an ordinary practitioner would have recognized that the results optimizable variables of time, product amount and sequence length could be adjusted to maximize the desired results. As noted in *In re Aller*, 105 USPQ 233 at 235,

> More particularly, where the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation.

Routine optimization is not considered inventive and no evidence has been presented that the selection of specific amplicon sizes was other than routine, that the products resulting from the optimization have any unexpected properties, or that the results should be considered unexpected in any way as compared to the closest prior art.

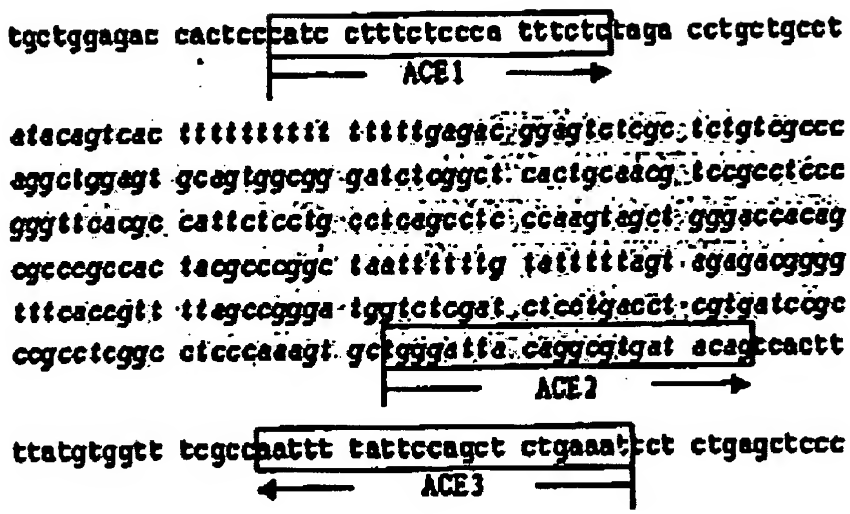


**Fig. 1. Design of primers for real-time PCR detection of ACE gene insertion/deletion polymorphism. The sequences and relative position of primers ACE1, ACE2, and ACE3 were boxed. The gray-filled region was the intron 16 sequences of ACE gene.**

Considering that this is a known region of interest of limited length (see Figure inserted above, Lin, Figure 1), the primers must be selected within this specific region, including the flanking primers and particularly the insertion specific primer site (ACE2 above). It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have designed primers within these defined regions to obtain amplicons of a predetermined length. The process of designing primers would have been obvious to one of ordinary skill in the art at the time the invention was made in order to achieve the predictable result of obtaining an amplicon of the desired size.

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to adjust and optimize the location of the primers taught by Lin and Hiratsuka, including those disclosed within the instant application and disclosed generally by Soubrier in order to achieve the optimal resolution of the genotypes of the insertion and/or

deletion alleles of the ACE gene, including amplicon sizes which meet the limitation of the method as claimed.

**(10) Response to Argument**

***Claims 2, 40-48, 50 and 67-72 are rejected under 35 U.S.C. 103 as being unpatentable over Lindpainter in view of Lin***

Applicant's arguments filed May 8, 2007 have been fully considered but they are not persuasive. When addressing the combination of these references together, Applicant asserts "Lin, like Applicant, use a flanking primer pair and an internal, fragment specific primer. However, the implementation and the result of this primer strategy is vastly different". Applicant also states that "despite the similarities in primer design, the difference in product number is a function of implementation". Additionally, it is noted "Appellants' method produces two amplicons from the I-allele; whereas Lin produces only one single amplicon. Specifically, Appellants produce one amplicon from the flanking primers (ACE1 and ACE3 of Lin) and another amplicon from the fragment-specific primer and one flanking primer (ACE2 and ACE3 of Lin). By contrast, Lin produces only the latter" (p. 13 of brief).

These arguments, taken as a whole are not persuasive. Applicant is correct that Applicant and Lin share very similar primer design, however it is unclear how a "function of implementation" could result in the absence of a PCR product that would be expected, necessarily, to be present using the three primer strategy taught by Lin. As noted by Applicant, Lin teaches the detection of two 'short' amplicons, corresponding to the I allele (65 bp) and D allele (84 bp) respectively. However, Lin does not explicitly address or discuss the presence of

the third amplicon corresponding to the product of the ACE1 and ACE3 primers, when the insertion region is present. Instead, Lin focuses attention on the products of the ACE1 and ACE3 reaction in samples where the insertion is not present (deletion allele) and detects the insertion fragment using an insertion specific primer, ACE2, in combination with the third flanking primer, ACE3.

While it is noted that Lin does not specifically address the presence of a third amplicon, the method of claim 1 and 67 does not require the detection of three amplicons in all genotypes. Three amplicons are only necessary for detection in heterozygous samples. Furthermore, it is also noted that Lin does teach the detection of three peaks in the melting curve analysis, as depicted at Figure 3A, corresponding to a peak for the I/I homozygotes, a peak for the D/D homozygotes and a peak for the I/D heterozygotes. Therefore, the genotype of the sample can be determined through an analysis of the number of peaks present in the detection of the amplification reaction in samples that are not heterozygous.

While it remains unclear why the third amplicon (product of flanking primers when the insertion is present) that should be present in the amplification reaction is not detected, a supporting reference discussed in Lin, Hiratsuka, (as applied in a new grounds of rejection in view of Lin as recited above) teaches that flanking primers that bind in nearly the exact same region as taught by Lin do in fact produce a larger insertion-specific product 387 bp in length in the presence of the insertion polymorphism. In samples where the insertion is not present (the deletion allele), the product of these flanking primers produces a product that is 85 bp in length, nearly identical to the D amplicon produced by Lin. Furthermore, Hiratsuka teaches a similar melting curve analysis of the I and D amplicons using the flanking primers and upon detecting

the 'long' I allele, the melting curve reflects an extended melting process for the 387 bp amplicon, comprising melting peaks at 80.7 °C, 85.8°C and 88.8°C. Therefore, in combination with the teachings of Lin, a characteristic number of amplicons is present in samples depending on a homozygous or heterozygous genotype. This combination is taught in the new grounds of rejection stated above.

Regarding Lindpainter, Applicant asserts "Lindpainter does not provide what Lin lacks. As discussed above, Lindpainter requires the use of two PCR amplifications and four primers in order to determine every ACE genotype. Like Lin, Lindpainter does not produce a distinct number of amplicons for each genotype" (p. 14 of brief). In response it is noted that Lindpainter does teach two amplicons for the I allele. In the first PCR amplification, using the primers flanking the Alu insertion region of the ACE gene (hace3s and hace3as), Lindpainter produces an 'insertion' amplicon of 597 bp. In the second amplification reaction, using the insertion specific primer hace5a and an additional primer, an insertion specific amplicon of 335 bp is produced. Therefore, Lindpainter does provide two amplicons for the I allele and one amplicon for the D allele. What Lindpainter does not teach is simultaneous amplification of the region using all four primers together in the same reaction. As noted in the 103 rejection stated above, Lin teaches a method for amplification using three primers together in the same reaction. Considering the potential for high throughput analysis using the real-time and multiplexed method taught by Lin and the significant time saving provided by multiplexing the amplification using the three primers simultaneously, it would have been obvious to extend the method of

Lindpainter or Lin to achieve two I amplicons and a single D amplicon in a single amplification reaction.

***Claims 49 and 54-59 are rejected under 35 U.S.C. 103 as being unpatentable over Lindpainter in view of Lin, Soubrier and Buck. Claim 66 is rejected under 35 U.S.C. 103 as being unpatentable over Lindpainter in view of Lin, van Bockxmeer, Soubrier and Buck.***

Applicant asserts that the genotyping strategy of Soubrier is significantly different from the method claimed and that it is substantially similar in format to the method taught by Lindpainter and does not suggest simultaneous amplification and does not remedy the deficiencies of Lindpainter and Lin as discussed previously.

Regarding Buck, Applicant asserts that the combination of Buck with Soubrier, Lindpainter and Lin is improper. Applicant states that Buck is concerned with testing the effectiveness of sequencing primers and "the use of sequencing primers cannot be equated with the use of PCR primers" (p. 17-18 of remarks).

These arguments are not persuasive. First, regarding Buck, Applicant's argument that sequencing primers cannot be equated with PCR primers creates a smokescreen. A primer is extended with a DNA polymerase in the same manner in sequencing reactions and in PCR amplifications. The same issues of hybridization of the primer, sequence specificity and primer extension apply in sequencing and PCR. In PCR, two primer hybridization and extension events occur, while in sequencing a single primer is extended. However, the teaching of Buck that a primer would be expected to function regardless of sequence is equally relevant to issues of sequencing as it is to the instant case where primers are selected specifically for PCR. The

combination asserts that it is obvious to choose primers within the set sequence of the angiotensin converting enzyme. Therefore, the combination is proper.

Finally, regarding Soubrier, the reference is relied upon for specific teaching of the sequences disclosed in SEQ ID NOs:1-3 and not specifically for the method taught by Soubrier. However, as noted by Applicant, Soubrier teaches a method that is very similar to the methods discussed above and includes amplification using primers flanking the polymorphic region and also teaches the use of and insertion specific primer. Therefore, the combination is proper and Applicant's arguments regarding Soubrier are not persuasive.

***Claims 62-65 are rejected under 35 U.S.C. 103 as being unpatentable over Lindpainter in view of Lin and van Bockxmeer.***

Applicant traverses these rejections on the grounds that the combination of Lindpainter and Lin does not render obvious Applicant's claimed method for ACE genotyping. Regarding the limitations of the rejected claims, Applicant states that van Bockxmeer is relied upon "to demonstrate that the prior art recognized an association between an individual's ACE genotype and their likelihood of developing certain cardiovascular diseases. For this, there is no dispute" but that this does not remedy the deficiency of Lindpainter and Lin.

These arguments are not persuasive. While Applicant's statement regarding the van Bockxmeer reference and the prior art teaching of association between ACE genotype and predisposition to developing cardiovascular disease is correct, Applicant's arguments regarding Lindpainter and Lin are not persuasive for the reasons stated previously.

***Claims 2, 40-48, 50 and 60-70 under 35 U.S.C. 103 as being unpatentable over Teranishi in view of Lin.***

Applicant traverses this rejection for substantially the same reasons and with the same basic arguments as applied for Lindpainter and Lin. These arguments are not persuasive for the same basic reasons as applied regarding Lindpainter in view of Lin as noted above.

Teranishi teaches amplification and genotyping of a polymorphic insertion in the angiotensin converting enzyme gene. The method comprises amplification of the ACE gene using flanking primers and an insertion specific primer. Effectively, Teranishi teaches a two step, three primer amplification. When Teranishi is combined with the three primer, single amplification method taught by Lin, a method that comprises two insertion specific amplicons together with a deletion specific amplicon is achieved. The result of this combination of references yields genotype analysis comprising two amplicons in I allele homozygotes, a single amplicon in D allele homozygotes and three amplicons in I/D heterozygotes. This panel of amplification products meets the limitations of the method as claimed. Therefore, these rejections are maintained.

For the above reasons, it is believed that the rejections should be sustained.

Respectfully submitted,

Stephanie K. Mummert

Stephanie Mummert

Examiner, Art Unit 1637

Director TC 1600
(acting)

Conferees:

Gary Benzion, SPE Art Unit 1637

Peter Paras, SPE Art Unit 1632

Jeffrey Fredman, Primary Examiner, Art Unit 1637

Gary Benzion

GARY BENZION, PH.D
SUPERVISORY PATENT EXAMINER
TECHNOLOGY CENTER 1600

Pete Paras

PETER PARAS, ~~JR.~~
SUPERVISORY PATENT EXAMINER
TECHNOLOGY CENTER 1600

JEFFREY FREDMAN
PRIMARY EXAMINER
9/17/07